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IN SILICO EVALUATION OF HEXADECANOIC ACID AS A POTENTIAL GABAERGIC AND SEROTONERGIC MODULATOR: MOLECULAR DOCKING AND ADMET ANALYSES

AVALIAÇÃO IN SILICO DO ÁCIDO HEXADECANOICO COMO POTENCIAL MODULADOR GABAÉRGICO E SEROTONINÉRGICO: DOCKING MOLECULAR E ANÁLISES ADMET

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ABSTRACT

Anxiety disorders are major contributors to global mental health impairment, demanding new therapeutic strategies with greater efficacy and fewer adverse effects. This study investigated the anxiolytic and antidepressant potential of hexadecanoic acid (HA), a saturated fatty acid, using in silico methods. Pharmacokinetic and toxicological predictions (pkCSM, ADMETlab 3.0, SwissADME, Molinspiration) indicated high intestinal absorption, CYP3A4 metabolism, and low toxicity. Docking simulations (CB-Dock2) were performed with $GABA_A$ (α1, α2, α3, α5) and $5\text{-}HT_{1A}$ (chains D and E) receptors. Diazepam, flumazenil, and fluoxetine served as reference ligands. Structural analyses (PyMOL, Discovery Studio) revealed significant HA affinity for both receptors, with stable interactions mainly via hydrophobic and van der Waals forces. Despite limited blood-brain barrier permeability, HA demonstrated potential as a modulator of GABAergic and serotonergic pathways. These findings support further in vivo studies and formulation strategies to improve CNS bioavailability.

Keywords: *Hexadecanoic Acid; ADMET, Molecular Docking; 5-HT1 Receptor; Anxiolytics; $GABA_A$.*

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RESUMO

Os transtornos de ansiedade contribuem significativamente para o comprometimento da saúde mental global, exigindo novas estratégias terapêuticas com maior eficácia e menos efeitos adversos. Este estudo investigou o potencial ansiolítico e antidepressivo do ácido hexadecanoico (AH), um ácido graxo saturado, utilizando métodos in silico. As previsões farmacocinéticas e toxicológicas (pkCSM, ADMETlab 3.0, SwissADME, Molinspiration) indicaram alta absorção intestinal, metabolismo via CYP3A4 e baixa toxicidade. Simulações de acoplamento molecular (CB-Dock2) foram realizadas com os receptores GABA (α1, α2, α3, α5) e 5-HT1A (cadeias D e E). Diazepam, flumazenil e fluoxetina foram utilizados como ligantes de referência. As análises estruturais (PyMOL, Discovery Studio) revelaram afinidade significativa do AH por ambos os receptores, com interações estáveis principalmente por meio de forças hidrofóbicas e de van der Waals. Apesar da permeabilidade limitada da barreira hematoencefálica, o HA demonstrou potencial como modulador das vias GABAérgicas e serotoninérgicas. Esses achados justificam a realização de mais estudos in vivo e o desenvolvimento de estratégias de formulação para melhorar a biodisponibilidade no SNC.

Palavras-chave: *Ácido Hexadecanoico; ADMET; Acoplamento molecular; Receptor 5-HT1; Ansiolíticos; GABA$_A$.*

1 INTRODUCTION

Central nervous system (CNS) disorders encompass a diverse group of neurological diseases that are becoming increasingly common worldwide. Among the main neuropsychiatric conditions are anxiety, insomnia, depression, epilepsy, dementia and chronic pain (Hernández-Sánchez *et al.*, 2023). These conditions often coexist, making the diagnosis, clinical approach and treatment more complex (Husain *et al.*, 2023).

The pathophysiological mechanisms associated with central nervous system (CNS) disorders can involve various neurotransmitters, such as serotonin (5-HT), dopamine (DA), glutamate and γ-aminobutyric acid (GABA) (Li *et al.*, 2022; Oshaghi *et al.*, 2023). In general, it is observed that the imbalance between excitatory and inhibitory neurotransmitters plays a crucial role in these clinical conditions, directly affecting *functions* such as cognition, emotions, memory and learning (Teleanu *et al.*, 2022). Thus, the pharmacological treatment of CNS disorders often requires complex approaches, especially when two or more disorders coexist (Koren *et al.*, 2024).

However, it is important to note that drugs used to treat CNS conditions are often associated with significant adverse effects. These include the potential for addiction, sedation, amnesia, cognitive impairment, sexual dysfunction and anticholinergic effects, which can impair adherence to treatment (Koren *et al.*, 2024). Faced with these challenges, the search for new compounds or substances with promising neuropharmacological properties and lower incidence of side effects remains a priority in medical and pharmacological research (Diniz *et al.*, 2019).

Hexadecanoic acid (HA), or palmitic acid, is a saturated fatty acid widely present in the human diet, found in various sources of animal and plant origin, such as vegetable oils, meat and dairy products (Ojha *et al.*, 2024). Although naturally present in the body, recent studies have raised

concerns about its impact on metabolic and cardiovascular health, due to its association with inflammatory processes, insulin resistance and endothelial dysfunction (Cui *et al*., 2021; Wang *et al*., 2021)

In addition to these negative effects, palmitic acid also plays an important role in brain functions, suggesting its influence on the modulation of behavior and mood disorders such as anxiety. Fatty acids are essential for cell membrane integrity, intracellular signaling, and modulation of neurotransmitter receptors. Moreover, HA can directly impact these functions (Lama *et al*., 2022). Specifically, palmitic acid has the potential to interact with GABAergic and glutamatergic neurotransmission pathways, which are fundamental in the regulation of anxiety (Kiecolt-Glaser *et al*., 2011; Lama *et al*., 2022). In addition, studies suggest that fatty acids can modulate the expression of serotonergic receptors, such as 5-HT1, which play a central role in regulating mood and the stress response (Vasović *et al*., 2023; Yin *et al*., 2018).

Despite advances in understanding the effects of fatty acids on the central nervous system, few studies have explored the therapeutic potential of HA as an anxiolytic agent, especially with regard to its interaction with serotonergic receptors, such as 5-HT1. Serotonin (5-HT) is a critical neurotransmitter in the regulation of anxiety and depression disorders, and receptors such as 5-HT_{1A} are directly involved in modulating the stress response and controlling anxious behaviors (Albert *et al*., 2014). In addition, the use of computational tools, such as molecular docking simulations and ADMET analyses, has proven to be a promising approach to identifying compounds with pharmacological potential, reducing costs and time associated with traditional experimental methods (DELANO, 2015; Macindoe *et al*., 2010).

The *in silico* approach allows for the prediction of pharmacokinetic and pharmacodynamic properties. These include blood-brain barrier permeability, plasma protein binding, and interactions with metabolizing enzymes, such as cytochrome P450. These properties are essential for developing safe and effective drugs(Pires *et al*., 2015). In addition, molecular docking makes it possible to identify binding sites and assess the affinity of HA with specific receptors, such as $GABA_A$ and 5-HT_{1A}, providing insights into its mechanism of action at a molecular level.

This study aims to evaluate the therapeutic potential of hexadecanoic acid (HA) as a possible anxiolytic agent. Through in silico ADMET and molecular docking studies, we will explore the pharmacokinetic profile, the interaction with the $GABA_A$ e 5-HT_{1A} receptores and the pharmacological viability of HA, contributing to the understanding of its mechanism of action and paving the way for future experimental investigations. This approach could open new avenues for developing fatty acid-based therapies, providing alternatives to conventional treatments for anxiety disorders, which often have significant side effects and variable response rates.

2 METHODOLOGY

2.1 *IN SILICO* APPROACH

2.1.1 Preparation of binders

The three-dimensional (3D) conformers of the compound hexadecanoic acid (PubChem ID: 22311), the standard drug Diazepam - DZP (PubChem ID: 3016), Flumazenil - FLU (PubChem ID: 3373) and Fluoxetine (PubChem ID: 3386) were retrieved in SDF format from the PubChem chemical database (https://pubchem.ncbi.nlm.nih.gov/) (Kim *et al*., 2021), accessed on April 27, 2025.

2.1.2 Physicochemical properties and drug similarity

The physicochemical properties and drug similarity of the compound were evaluated using widely validated computational tools. The analysis of physicochemical properties was carried out using the Molinspiration software, while drug similarity was screened using the SwissADME online tool (available at: http://swissadme.ch/) (Nunes da Rocha *et al*., 2022). Among the parameters calculated are the octanol-water partition coefficient (log P), molar refractivity, molecular weight, number of heavy atoms, number of hydrogen donors, number of hydrogen acceptors and the number of violations of the drug similarity rules.

In addition, molecular surface properties, such as molecular lipophilicity potential (MLP) and polar surface area (PSA), were visualized in the compound's three-dimensional (3D) structure using Molinspiration Galaxy 3D Structure Generator (version 2021.01 beta) (Nadeem *et al*., 2016). These parameters are critical for predicting cell permeability and the ability to cross biological barriers, such as the blood-brain barrier.

Other drug similarity criteria, including the Pfizer Rules (3/75), the GSK Rules (4/400), the Pfizer Golden Triangle and the Quantitative Estimation of Drug Similarity (QED), were analyzed using the ADMETlab 3.0 platform (Nunes da Rocha *et al*., 2022). These metrics are widely used to assess the viability of molecules as drug candidates, considering parameters such as solubility, permeability and toxicity.

2.1.3 Absorption, distribution, metabolism, excretion and toxicity prediction

Hexadecanoic acid was subjected to a detailed analysis of its ADMET properties (absorption, distribution, metabolism, excretion and toxicity) (Basharat *et al*., 2021) to evaluate its potential as a drug candidate (Feng *et al*., 2015; Fukunishi *et al*., 2014). The *in silico* evaluation was carried out

using the pkCSM software (Kavaliauskas *et al.*, 2020), which makes it possible to predict key parameters such as intestinal permeability, plasma protein binding, blood-brain barrier (BBB) permeability, metabolism mediated by cytochrome P450 enzymes and toxicity (Pires *et al.*, 2015) . These predictions are essential for understanding the viability of hexadecanoic acid as a pharmacologically active compound, with a focus on minimizing potential adverse effects and optimizing its pharmacokinetic properties (Daina and Zoete, 2016).

2.1.4 Toxicity

The toxicity profile of hexadecanoic acid (HA) was assessed using the ADMETlab platform, considering a total of 13 predictive parameters. The toxicity profile included LD50, toxicity class, hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, rERG I Inhibitor, rERG II inhibitor, skin sensitization, DÍLI, toxicity AMES, acute oral toxicity in Rats.

2.1.5 Protein Selection and Preparation

For the molecular docking studies, the α1, α2, α3 and α5 subunits of the human $GABA_A$ receptor were selected, based on experimental data and evidence from the literature associating these subunits with anxiolytic and sedative modulation (Bhuia *et al.*, 2023; Liao *et al.*, 2022; Luscher *et al.*, 2023; Rudolph and Knoflach, 2011; Vollenweider *et al.*, 2011). In addition, the serotoninergic receptor $5\text{-}HT_{1A}$ (D and E chain) was included, considering its central role in mood regulation and the pathophysiology of depression (Savitz *et al.*, 2009) .

The three-dimensional structures of the target proteins were obtained from the PDB and UniProt databases, with the following identifications:

$GABA_A$ α1: PDB ID 6HUJ / UniProt ID P14867
$GABA_A$ α2: PDB ID 6HUG / UniProt ID P47869
$GABA_A$ α3: PDB ID 6HUJ / UniProt ID P34903
$GABA_A$ α5: PDB ID 7QNE / UniProt ID P31644
$5\text{-}HT_{1A}$: PDB ID 3GWW (chains D and E)

The BLAST tool, provided by NCBI, was used to verify the identity and alignment of the sequences (Boratyn *et al.*, 2012). The structures were prepared for docking according to the recommendations of the platforms used.

2.1.6 Definition of active cavities (GABA $_A$ α1, α2, α3, α5 and 5-HT$_{1A}$)

The active sites of the proteins were identified using CB-Dock2, a platform that combines cavity prediction with pocket-guided docking, based on the CurPocket algorithm. The selected cavities were determined taking into account the volume (Å³), the position of the geometric center and the contact residues involved.

2.1.7 GABA$_A$ receptor, α1 subunit

Volume: 1576 Å³;

Geometric center: (-6.97, 10.62, -1.01);

Main residues: His129, Cys166, Pro167, Met168, His169, Glu171, Asp172, Pro174, Met175, Asp176, Ala177, His178, Ala179, Pro181, Lys183, Leu221, Asp226, Ser227, Gly228, Ile229, Val230, Val239, Thr241, His243, Lys247, Arg248, Ile250, Tyr442, Leu443, Arg445, Glu446, Pro447, Gln448, Leu449, Lys450, Ala451, Pro452.

2.1.8 GABA$_A$ receptor, α2 subunit

Volume: 745 Å³

Geometric center: (2.34, -2.91, 1.92)

Main residues: Cys166, Pro167, Met168, His169, Glu171, Asp172, Pro174, Met175, Asp176, Ala177, His178, Ser179, Pro181, Leu221, Ser224, Ile225, Gly226, Glu228, Thr241, His243, Lys247, Arg248, Ile250, Tyr440, Leu441, Arg443, Glu444, Pro445, Val446, Leu447, Gly448, Val449.

2.1.9 GABA$_A$ receptor, α3 subunit

Volume: 1199 Å³

Geometric center: (13.72, 15.75, -12.97)

Main residues: Leu14, Gly15, Leu17, Phe18, Leu19, Asn21, Ile22, Glu196, Asp197, Ala335, Trp340, Ala343, Val344, Ala347, Phe348, Ile469, Phe470, Val473, Tyr474, Thr477, Tyr478, Arg481.

2.1.10 GABA$_A$ receptor, α5 subunit

Volume: 1681 Å³

Geometric center: (0.44, 6.35, 1.68)

Main residues: Met175, Gln176, Leu177, Glu178, Asp179, Asp183, Ala184, His185, Tyr316, Arg451, Glu452, Pro453, Val454, Ile455, Lys456.

2.1.11 5-HT_{1A} receptor (PDB ID: 3GWW, chains D and E)

Volume: 658 Å³

Geometric center: (22.04, 22.11, 25.95)

Main residues: Leu25, Gly26, Leu29, Arg30, Val33, Gln34, Tyr107, Tyr108, Ile111, Gly242, Ile245, Ala246, Gly249, Gln250, Phe253, Ala319, Phe320, Leu400, Asp401, Asp404, Phe405, Thr409, Trp467, Tyr471, Ile475.

The diverse chemical composition of the residues in the binding pockets allows for the formation of multiple non-covalent interactions - including hydrophobic, hydrogenic and electrostatic - favoring the formation of stable complexes between ligands and receptors.

2.1.12 Docking protocol and non-bond interactions

The molecular docking between hexadecanoic acid (HA) and the $GABA_A$ (subunits α1, α2, α3 and α5) and 5-HT_{1A} (chains D and E) receptors was performed using the CB-Dock2 platform, which integrates cavity detection with docking simulations based on AutoDock Vina (Preethi *et al.*, 2024). In this approach, potential binding pockets are automatically identified based on the three-dimensional topology of the receptor, allowing the exploration of both canonical pharmacological sites and possible alternative interaction regions.

Diazepam and flumazenil were used as reference ligands because they are well-established modulators of the benzodiazepine binding site of the $GABA_A$ receptor, acting respectively as a positive allosteric modulator and a competitive antagonist. Their inclusion allowed comparison of binding affinity values and interaction patterns obtained in the docking simulations, serving as pharmacological benchmarks for the GABAergic system (Chang *et al.*, 2024).

Fluoxetine, although primarily recognized as a selective serotonin reuptake inhibitor (SSRI) acting on the serotonin transporter (SERT), was included as a serotonergic reference compound due to its indirect modulation of serotonergic neurotransmission and reported interactions with serotonin receptor systems, including 5-HT_{1A} signaling pathways. Therefore, its inclusion aimed to provide a comparative parameter for serotonergic ligand-receptor interactions within the docking analysis (De Araujo *et al.*, 2025).

Structural analyses and identification of non-covalent interactions were carried out using PyMOL (version 1.4.7) (Daina *et al.*, 2017) and Biovia Discovery Studio Visualizer 2021. The tools enabled three-dimensional visualization of the protein-ligand complexes and characterization of the molecular interactions involved, such as hydrogen bonds, hydrophobic interactions, pi-pi stacking, pi-alkyl, among others. All the software used is freely available for academic use.

3 RESULTS

3.1. PHARMACOKINETIC ANALYSIS AND DRUG-LIKENESS

Hexadecanoic acid (HA), a saturated fatty acid widely present in various natural sources, has attracted increasing interest due to its potential in various applications, including the pharmaceutical industry (Ojha *et al.*, 2024). In order to evaluate the pharmacokinetic and toxicological profile of HA and explore its viability as a drug candidate, we carried out an *in silico* study using ADMET prediction tools.

Tables 1 and 2 present the physicochemical properties and drug similarity of the HA compound, with a focus on HA. The analysis was carried out using the Molinspiration and SwissADME tools, which are widely used to screen drug candidates.

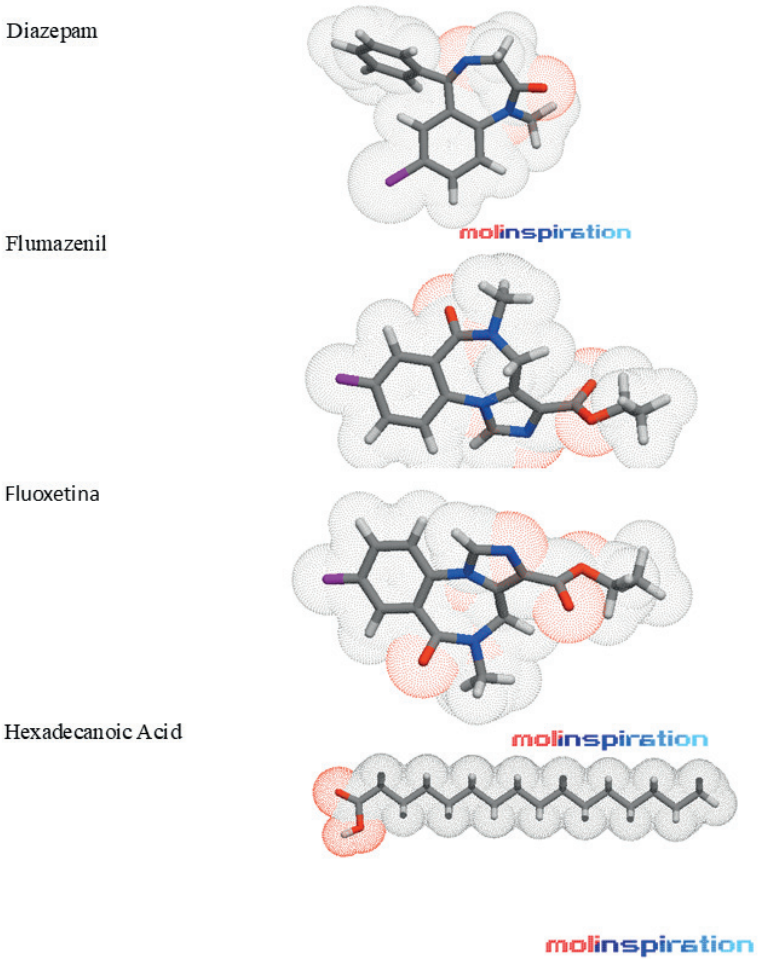
Table 1 - Physicochemical properties of the HA compound analyzed by Molinspiration.

Compound	MW (g/mol)	PSA (Å²)	N-atoms	Volume (Å³)	nON	nOHNH	Nviolations	Nrotb	milogP
DZP	284.75	32.67	20	246.54	3	0	0	1	2.74
FLU	303.29	64.44	22	257.21	6	0	0	3	0.86
FLX	309.33	21.26	22	275.13	2	1	0	7	4.53
HA	256.43	37.30	18	291.42	2	1	1	14	7.09

DZP - Diazepam FLU - Flumazenil; FLX- Fluoxetina; HA - Hexadecanoic acid.

HA presented a molecular weight (MW) of 256.43 g/mol (Figure 1), which is within the limit established by Lipinski's Rule of Five (≤ 500 g/mol), indicating a favorable potential for oral absorption. The topological polar surface area (TPSA) was 37.30 Å², a relatively low value, suggesting good permeability through biological membranes, including the blood-brain barrier.

Figure 1 - Molecular structure of Diazepam (DZP); Flumazenil (FLU); Fluoxetine (FLX); Hexadecanoic acid (HA). generated by the Molinspiration tool. The carboxyl group (-COOH) is highlighted in red, while the long hydrocarbon chain indicates its high lipophilicity.



Source: Author's own work.

When comparing HA with standard CNS drugs used as controls - diazepam (DZP), flumazenil (FLU), and fluoxetine (FLX) - it was observed that HA has a molecular weight slightly lower than that of DZP (284.75 g/mol), FLU (303.29 g/mol), and FLX (309.33 g/mol). This may favor faster absorption and distribution properties. Similarly, the TPSA value of HA (37.30 Å²) is intermediate among the controls, being higher than that of FLX (21.26 Å²) but lower than FLU (64.44 Å²), which supports its potential for blood-brain barrier penetration.

In terms of lipophilicity, HA exhibited a milogP value of 7.09, significantly higher than those of DZP (2.74), FLU (0.86), and FLX (4.53). This high logP suggests that HA is markedly more lipophilic, favoring partition into lipophilic tissues such as the central nervous system (CNS), although it may reduce aqueous solubility. Despite presenting one violation of Lipinski's rules due to the elevated logP, highly lipophilic compounds can be advantageous for interaction with targets located in lipid-rich environments, such as G-protein coupled receptors (GPCRs) in neuronal membranes.

This is particularly relevant since the 5-HT1 receptor, which is involved in anxiety modulation, is embedded in a hydrophobic environment, suggesting that lipophilic molecules like HA could have favorable binding interactions (Giorgioni *et al.*, 2024). The serotonin (5-HT) system, critical for emotional and motivational processes, exerts its diverse physiological effects through the activation of multiple receptor subtypes (5-HT 1-7). Furthermore, the 5-HT receptors are the primary targets for drugs aimed at treating depression, anxiety, and impulsivity disorders, and growing evidence highlights their role in learning and memory (Roberts *et al.*, 2020).

Thus, the pharmacokinetic profile of HA, although marked by high lipophilicity, presents several characteristics that justify its continued evaluation as a candidate for CNS-targeted therapies.

The drug-likeness analysis carried out by SwissADME showed that hexadecanoic acid (HA) meets the criteria of the Lipinski, Egan and Ghose Rules, indicating a favorable profile for the development of oral drugs. These rules evaluate parameters such as molecular weight, lipophilicity (log P), number of hydrogen donors and acceptors, which are essential for the absorption and distribution of compounds in the body. However, HA did not meet Veber and Muegge's more stringent criteria, which additionally consider molecular flexibility and the number of rotational bonds, suggesting that structural optimizations may be necessary to improve its drug-likeness (Table 2).

Table 2 - Drug similarity properties of the DZP, FLU, FLX and HA compound by SwissADME.

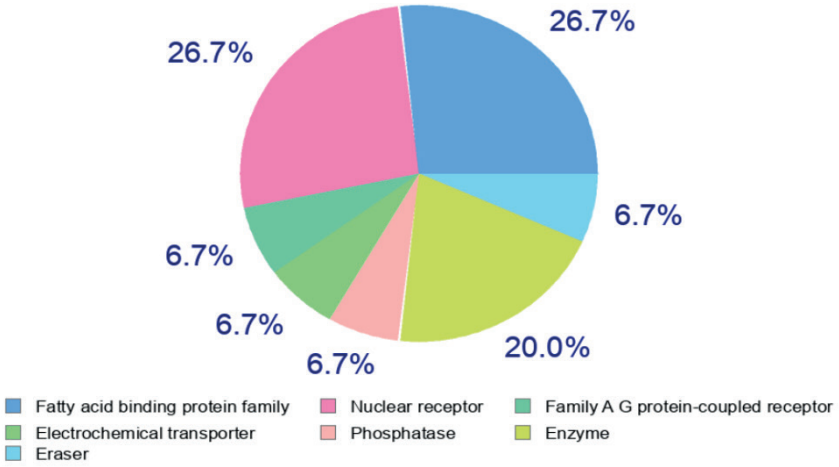
Compound	Verber	Drug linkeness				Bioavalability score
		Muegge	Lipinski	Egan	Ghose	
DZP	Yes	Yes	Yes	Yes	Yes	0.55
FLU	Yes	Yes	Yes	Yes	Yes	0.55
FLX	Yes	Yes	Yes	Yes	Yes	0.55
HA	No	No	Yes	Yes	Yes	0.55

** G protein-coupled receptors (GPCR), active if > 0; moderately active between -0.5 and 0; inactive if <-0.5*

3.2. Physicochemical properties and bioactivity

The bioavailability score was calculated, and Table 3 presents the HA bioactivity score in relation to different classes of molecular targets, using the Molinspiration tool. Bioactivity scores are a crucial metric for assessing the potential of a molecule to interact with relevant biological targets, such as G-protein-coupled receptors (GPCRs), ion channels, kinases, nuclear receptors, proteases and enzymes (Figure 2) (Pires *et al.*, 2015).

Figure 2 - Pie chart of the virtual sorting of the target class prediction.



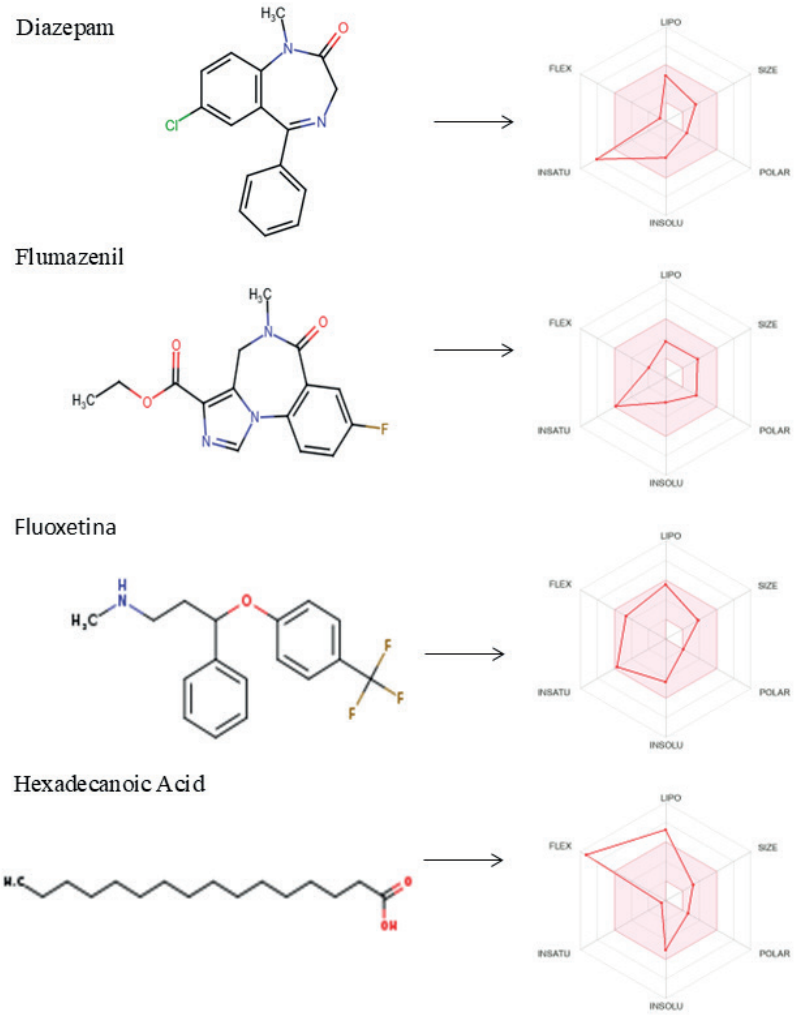
Source: Author's own work.

These targets are involved in various physiological and pathological processes, and interaction with them can determine the therapeutic efficacy of a compound (Albert *et al.*, 2014; Basak *et al.*, 2020; Diniz *et al.*, 2019). The assessment of the bioavailability and bioactivity of HA was carried out following validated methodologies, such as those described by Lipinski *et al.* (Lipinski, 2016) and Daina *et al.* (Daina *et al.*, 2017), which highlight the importance of computational tools in the screening of drug candidates.

3.3. Evaluation of pharmaceutical and medicinal chemistry

By performing a visual inspection on the bioavailability radar in Figure 3, it can be seen that HA is within the spectrum of ideal molecular size, lipophilicity, unsaturation, flexibility and polarization according to the criteria of Lipinski's rule, (Lipinski, 2016).

Figure 3 - Bioavailability radar plot representing predicted physicochemical parameters of DZP, FLU, FLX and HA. The pink area represents the optimum range for each parameter.



Source: Author's own work.

3.4. ADMET profile and toxicity

The ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties of hexadecanoic acid (HA) were predicted using the pkCSM tool in order to assess its pharmacological potential and toxicological safety profile (Table 3).

Table 3 - ADME and toxicity using the pkCSM tool.

		DZP	HA	FLU	FLX	Unit
Absorption						
Intestinal absorption (human)		97.42	92.00	93.567	91.371	(%Absorbed)
Distribution						
VDss (human)		0.365	-0.543	0.101	1.19	(log L/kg)
BBB Permeability		0.331	-0.111	-0.205	0,19	(log BB)
CNS Permeability		-1.397	-1.816	-3.015	-1.329	(log PS)
Metabolism						
CYP	2D6 substrate	No	No	No	No	Categorical (Yes/No)
	3A4 substrate	Yes	Yes	No	Yes	Categorical (Yes/No)
	2C19 inhibitor	Yes	No	No	Yes	Categorical (Yes/No)
	2C9 inhibitor	Yes	No	No	No	Categorical (Yes/No)
	2D6 inhibitor	No	No	No	Yes	Categorical (Yes/No)
	3A4 inhibitor	No	No	No	No	Categorical (Yes/No)
	1A2 inhibitor	Yes	No	Yes	Yes	Categorical (Yes/No)
Excretion						
Full compensation		0.294	1.763	0.758	0.694	Numeric (log ml/min/kg)

HA - Hexadecanoic acid; DZP - Diazepam; FLU - Flumazenil; FLX - Fluoxetina

The results indicate that HA shows high intestinal absorption in humans (92%), close to that of the reference drugs diazepam (97.42%) and flumazenil (93.57%), and higher than that of fluoxetine (91.37%). These data suggest good oral bioavailability, a fundamental criterion for the development of orally administered drugs (Pires *et al.*, 2015).

However, when we looked at distribution, HA showed a negative volume of distribution (VDss) (-0.543 log L/kg), indicating that it tends to remain more in the plasma than in the tissues. In comparison, the VDss of HA is lower than that of all the reference compounds, especially fluoxetine (1.19 log L/kg), suggesting limited peripheral distribution. This characteristic may be advantageous in therapeutic applications where penetration into the central nervous system (CNS) is to be avoided, such as in the treatment of metabolic or inflammatory disorders (Wang *et al.*, 2021).

However, when we looked at distribution, HA showed a negative volume of distribution (VDss) (-0.543 log L/kg), indicating that it tends to remain more in the plasma than in the tissues. In comparison, the VDss of HA is lower than that of all the reference compounds, especially fluoxetine (1.19 log L/kg), suggesting limited peripheral distribution. This characteristic may be advantageous in therapeutic applications where penetration into the central nervous system (CNS) is to be avoided, such as in the treatment of metabolic or inflammatory disorders.

In metabolism, HA is identified as a substrate of the CYP3A4 enzyme, one of the main enzymes involved in the biotransformation of drugs. Unlike DZP and FLX, HA does not significantly inhibit the main cytochrome P450 enzymes (CYPs), which reduces the risk of drug interactions. This aspect is crucial, as the inhibition of enzymes such as CYP2D6 and CYP2C9, observed in DZP and FLX, is often

associated with adverse effects (Cui *et al.*, 2021). HA is not a substrate of CYP2D6, nor does it inhibit CYP2C19, CYP2C9 or CYP1A2, indicating a low propensity to interfere with the metabolism of other compounds. In excretion, the log total clearance rate (clearance) of HA was 1.763 log ml/min/kg, the highest among the compounds analyzed, suggesting efficient elimination from the body, which may contribute to a favorable pharmacokinetic profile and reduce the risk of bioaccumulation (Table 3).

Also with regard to toxicity, the comparative data presented in Table 4 shows that hexadecanoic acid (HA) has an overall safety profile similar to or even superior to some reference drugs. Its estimated LD_{50} value was 900 mg/kg, higher than diazepam (48 mg/kg) and fluoxetine (248 mg/kg), indicating lower acute toxicity. Although it has a lower LD_{50} than flumazenil (1300 mg/kg), it still falls into toxicological class 4, considered to be of low oral toxicity.

Table 4 - Toxicity properties of hexadecanoic acid (HA) and reference compounds (DZP - Diazepam; FLU - Flumazenil; FLX - Fluoxetine) based on in silico predictions made by the Admetlab 3.0 tool.

Toxicity	Parameters	Test sample and reference drugs			
		DZP	**FLU**	**FLX**	**HA**
	LD_{50}	48 mg/kg	1300 mg/kg	248 mg/kg	900mg/kg
	Toxicity Class	2	4	3	4
	Hepatotoxicity	Inactive	Inactive	Inactive	Inactive
	Carcinogenicity	Inactive	Inactive	Inactive	Inactive
	Immunotoxicity	Inactive	Inactive	Inactive	Inactive
	Mutagenicity	Inactive	Inactive	Inactive	Inactive
	Cytotoxicity	Active	Inactive	Inactive	Inactive
	hERG I inhibitor	Inactive	Inactive	Inactive	Inactive
	hERG II inhibitor	Inactive	Inactive	Ative	Inactive
	Skin sensitization	Inactive	Inactive	Inactive	Ative
	DÍLI	Inactive	Inactive	Inactive	Inactive
	toxicity AMES	Inactive	Inactive	Inactive	Inactive
	Acute oral toxicity in rats	Inactive	Inactive	Inactive	Inactive

LD_{50}: Median lethal dose.

HA was classified as inactive for hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, acute oral toxicity and drug-induced liver toxicity (DILI), just like the reference compounds, demonstrating a promising toxicological profile. In addition, it showed no mutagenic activity in the Ames test, which indicates a low genotoxic risk.

A negative highlight was the active skin sensitization potential of HA, the only one of the compounds analyzed to show this effect. This indicates that the compound could cause allergic reactions or contact dermatitis in susceptible individuals. This finding reinforces the need for further studies in dermatological models to more accurately assess this risk before moving on to clinical phases.

With regard to cardiac toxicity, HA was classified as inactive for the inhibition of hERG I and II channels, HA demonstrated a better cardiac profile compared to fluoxetine, which was classified as active for hERG II inhibition, a potential risk for realization.

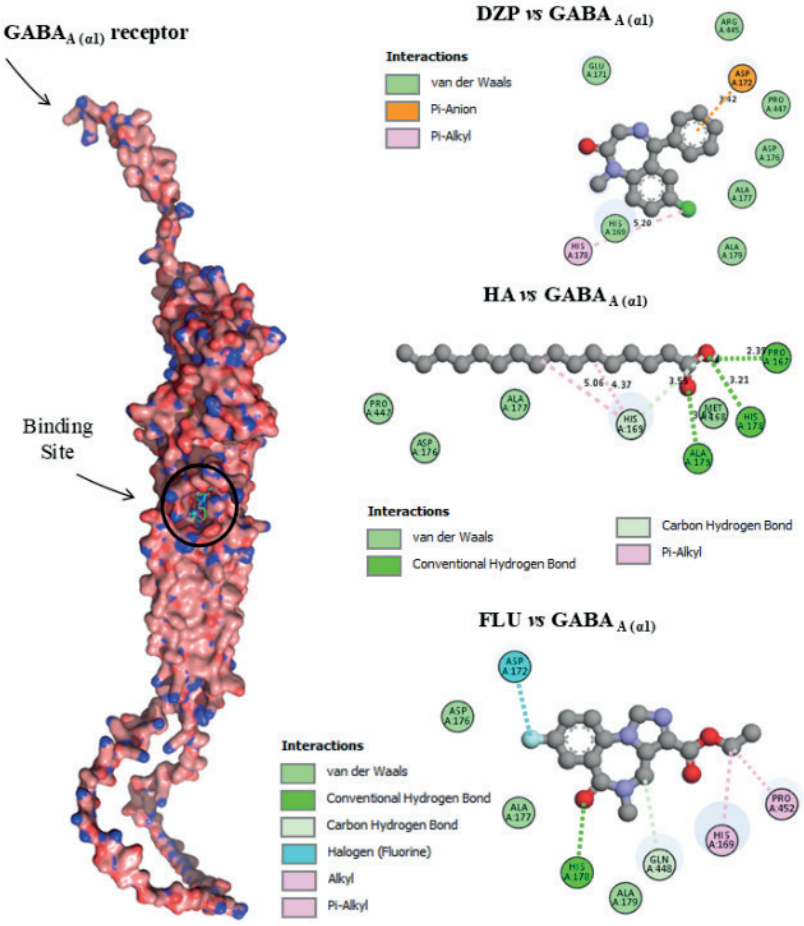
Finally, the HA compound also showed inactivity in terms of cytotoxicity, unlike diazepam, which showed cytotoxic activity, reinforcing its safety potential in cellular environments.

3.5. MOLECULAR DOCKING

3.5.1. HA, DZP and FLU with GABA $_A$ (α1) receptor interactions

DZP shows a high binding affinity of -6.7 kcal/mol, forming van der Waals, pi-anion, pi-alkyl bonds with Glu A:171, Arg A: 445, Pro A:447, Asp A: 176, Ala A: 177, Ala A: 179, His A: 169, Asp A: 172, His A: 178. HA demonstrated a binding affinity of -4.7 kcal/mol, forming van der Waals, conventional hydrogen bond, carbon-hydrogen bond and Pi-Alkyl bonds with His A: 169, Ala A: 179, His A: 178, Pro A: 167, Pro A: 447, Asp A: 176, Ala A: 177, Met A: 168. FLU showed a good bond affinity of -6.6 kacl/mol, forming van der Waals bonds, conventional hydrogen bond, carbon-hydrogen bond, halogen (fluorine), Alkyl, pi-alkyl with Gln A: 448, Hus A: 178, Ala A: 177, Asp A: 176, Asp A: 172, His A: 169, Pro A: 452 (Figure 4).

Figure 4 - Molecular interactions of diazepam (DZP) hexadecanoic acid (HA) and flumazenil (FLU) with the GABA$_A$ receptor (α1 subunit).

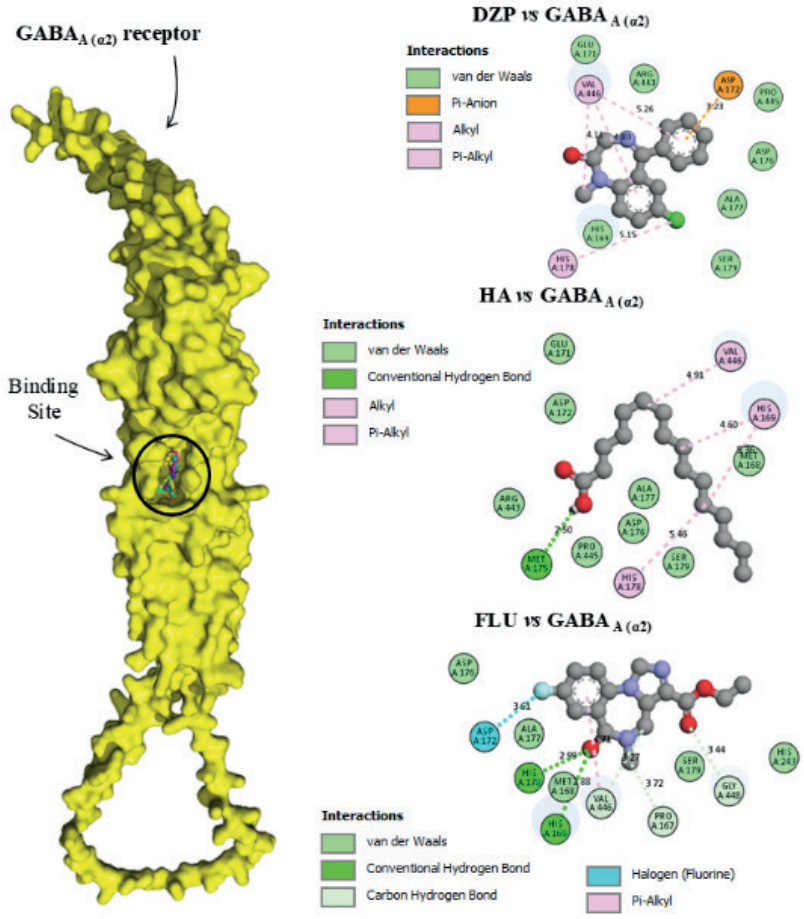


Source: Author's own work.

3.5.2. HA, DZP and FLU with GABA $_A$ (α2) receptor interactions

Molecular docking analysis showed that diazepam (DZP) had the highest binding affinity among the compounds evaluated, with a value of -7.0 kcal/mol. DZP established a pi-anion interaction with Glu A:171, and a hydrogen bond with Ser A:179, as well as relevant hydrophobic van der Waals and alkyl interactions with His A:169, Asp A:176, Arg A:443, Pro A:445 and Val A:446. Hexadecanoic acid (HA) showed an affinity of -4.7 kcal/mol, interacting mainly through conventional hydrogen bonds with Ser A:179 and His A:178, as well as van der Waals and alkyl interactions with Asp A:176, Glu A:171, Arg A:443, Met A:168 and Val A:446. The hydrophobic interactions favor the stabilization of the complex, even though its energy affinity is lower than that of DZP. Fluoxetine (FLX) exhibited intermediate affinity, with -6.5 kcal/mol, and formed a halogen (fluorine) bond with Pro A:167, two hydrogen bonds with His A:243 and Gly A:448, as well as significant pi-alkyl interactions with Val A:446 and Met A:178, suggesting a good fit in the active site of the α2 subunit of the GABA$_A$ receptor (Figure 5).

Figure 5 - Molecular interactions of diazepam (DZP), hexadecanoic acid (HA) and flumazenil (FLU) with the GABA$_A$ receptor (α2 subunit).

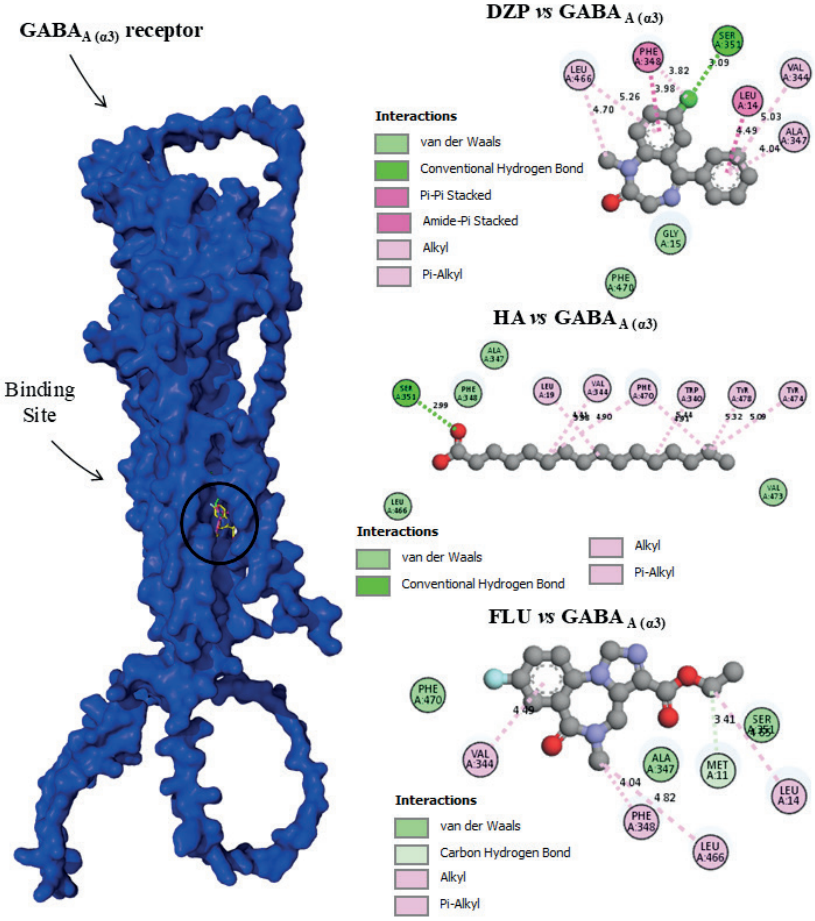


Source: Author's own work.

3.5.3. HA, DZP and FLU with GABA $_A$ (α3) receptor interactions

DZP showed a strong binding affinity of -6.8 kcal/mol, forming van der Waals bonds, conventional hydrogen bond, pi-pi stacked, amide-pi stacked, alkyl, pi-alkyl bonds with Phe A:470, Gly A:15, Ser A:351, Leu A:466, Val A:344, Ala A:347, Phe A: 348, Leu A: 14. HA showed a strong binding affinity of -6.0 kcal/mol, formed van der Waals, conventional hydrogen bond, Alkyl, pi-alkyl bonds with Leu A: 466, Val A:473, Phe A:348, Ala A:347, Ser A: 351, Leu A:19, Val A: 344, Phe A:344, Trp A:340, Tyr A:478, TyrA:474. FLU showed a strong binding affinity of -7.7 kcal/mol, formed van der Waals, carbon-hydrogen bonds, alkyl, pi-alkyl bonds with Phe A:470, Ala A:347, Ser A: 351, Met A: A11, Val A:344, Phe A:348, Leu A: 466, Leu A:14 (Figure 6).

Figure 6 - Molecular interactions of diazepam (DZP), hexadecanoic acid (HA) and flumazenil (FLU) with the GABA$_A$ receptor (α3 subunit).



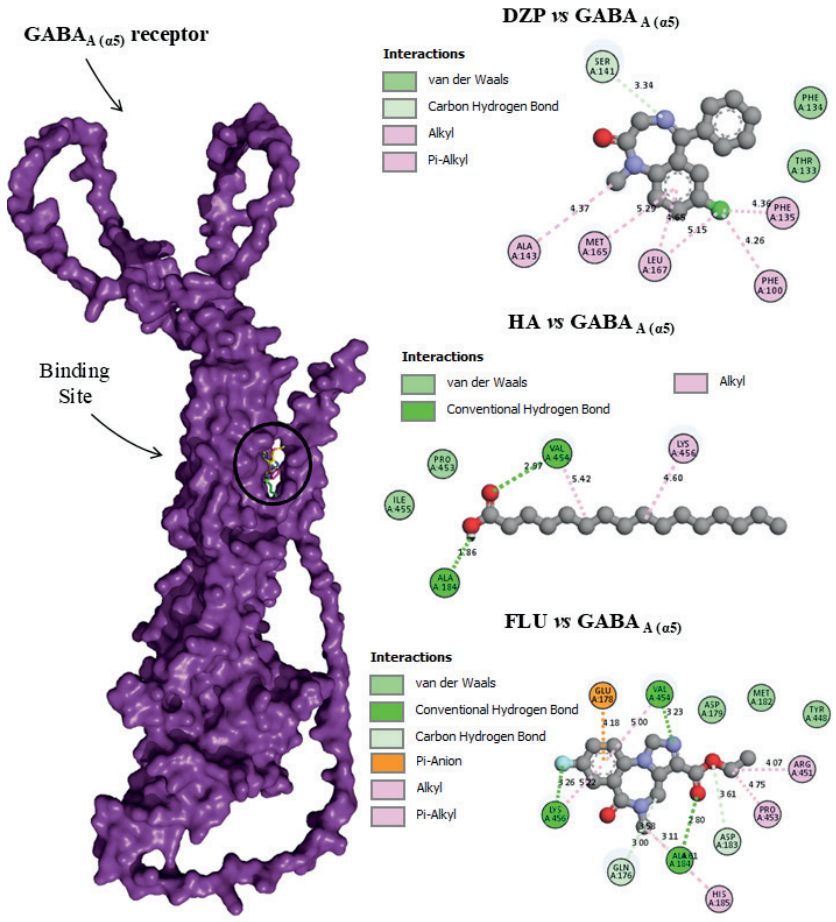
Source: Author's own work.

3.5.4. HA, DZP and FLU with GABA $_A$ (α5) receptor interactions

DZP showed a strong binding affinity of -6.0 kcal/mol, forming van der Waals, carbon-hydrogen bond, alkyl, pi-alkyl bonds with Thr A:133, Phe A:134, Ser A:141, Ala A:143, Met A:165,

Leu A:167, Phe A:100, Phe A: 135. HA showed bond affinity of -4.4 kcal/mol, forming van der waals bonds, conventional hydrogen bond, alkyl with Ile A: 455, Pro A:453, Ala A:184, Val A:454, Lys A:456. FLU showed a strong binding affinity of -6.7 kcal/mol, forming van der Waals bonds, conventional hydrogen bond, carbon-hydrogen bond, pi-anion, alkyl, pi-alkyl bonds with Gln A:176, Asp A:183, Lys A:456, Ala A:184, Val A:454, Asp A:179, Met A:182, Tyr A:448, Glu A:178, His A:185, Pro A:453, Arg A:451 (Figure 7).

Figure 7 - Molecular interactions of diazepam (DZP) hexadecanoic acid (HA) and flumazenil (FLU) with the $GABA_A$ receptor (α5 subunit).

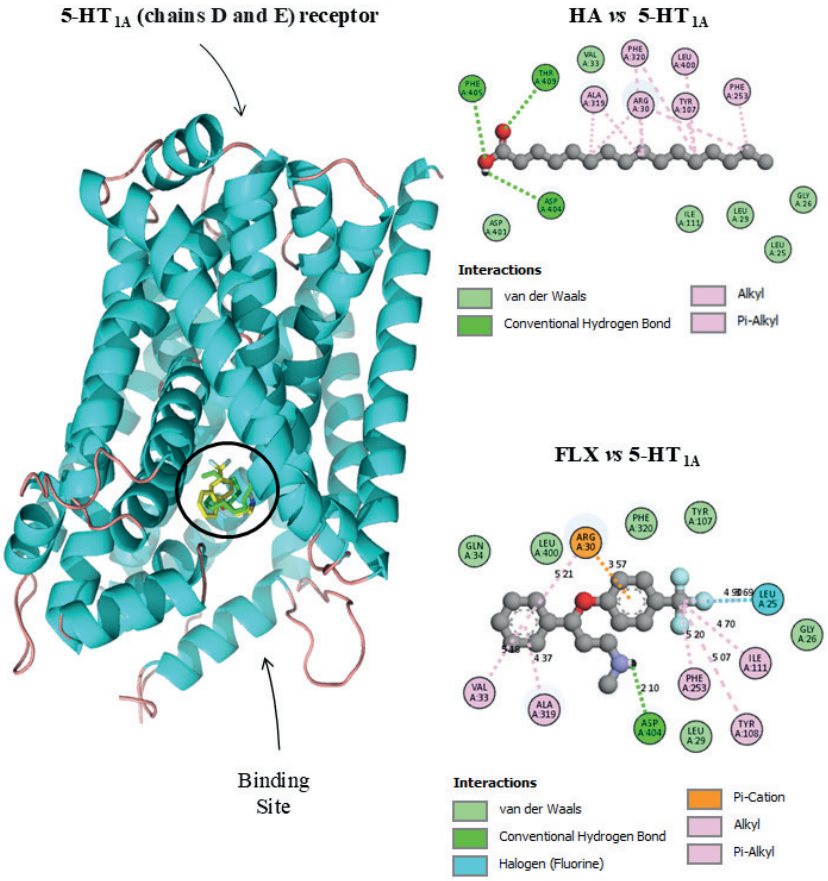


Source: Author's own work.

3.5.5. Hexadecanoic acid and FLX with $5HT_{1A}$ receptor interactions

Fluoxetine showed a strong binding affinity of -7.7 kcal/mol, forming van der Waals bonds, conventional hydrogen bond, halogen (fluorine), pi-cation, alkyl, pi-alkyl with Gln A:34, Leu A:400, Phe A:320, Tyr A:107, Gly A:26, Asp A:401, Leu A: 401 Leu A:29 Asp A:404, Leu A:25, Arg A:30, Val A:33, Ala A:319. HA showed an binding affinity of -4.7 kcal/mol, forming van der Waals, conventional hydrogen bond, alkyl, pi-alkyl bonds with Asp A:401, Ile A:111, Leu A: 29, Leu A:25, Gly A:26, Val A:33, Asp A:404, Phe A:405., Thr A:409, Ala, A:319, Phe A:320, Arg A:30, Leu A:400, Thr A:107, Phe A:253 (Figure 8).

Figure 8 - Molecular interactions of hexadecanoic acid (HA) and fluoxetine (FLX) with the 5-HT1A receptor (chains D and E).



Source: Author's own work.

5. DISCUSSION

5.1. PHYSICOCHEMICAL PROFILE AND DRUG-LIKENESS ASSESSMENT

Hexadecanoic acid (HA), a saturated fatty acid widely distributed in nature, has attracted increasing interest as a potential bioactive compound, particularly in applications targeting the central nervous system (CNS). In the present study, comprehensive in silico analyses were performed to evaluate its pharmacological viability, including physicochemical profiling, drug-likeness assessment, molecular bioactivity prediction, ADMET properties (absorption, distribution, metabolism, excretion, and toxicity), and molecular docking.

The results obtained using the Molinspiration, SwissADME, pkCSM, and AdmetLab 3.0 tools indicate that, despite the high lipophilicity of HA, the compound exhibits favorable pharmacokinetic and toxicological characteristics, such as appropriate molecular weight, good intestinal absorption, low acute toxicity, and absence of predicted genotoxic, carcinogenic, or hepatotoxic effects. Furthermore, the observed interactions with G protein-coupled receptors (GPCRs) suggest a potential role in modulating serotonergic pathways, which may be relevant to anxiety-related and behavioral disorders.

Drug-likeness is a fundamental criterion for predicting the "drug-like" characteristics of a chemical molecule during the early stages of drug discovery and development. It is evaluated through physicochemical properties that are closely related to pharmacokinetic behavior (Bhuia *et al*., 2023). One of the most widely used approaches for this purpose is the Lipinski's Rule of Five. According to this rule, a potential drug candidate should present a molecular weight of 500 g/mol or less, no more than five hydrogen bond donors (HBD), no more than ten hydrogen bond acceptors (HBA), and a lipophilicity (LogP o/w) of five or less (Lipinski, 2004). In the present study, hexadecanoic acid (HA) exhibited physicochemical properties generally compatible with drug-likeness criteria; however, it presented one violation of Lipinski's rule due to its elevated lipophilicity (milogP = 7.09), while the remaining parameters remained within the recommended limits. In addition, HA, DZP, and FLU showed physicochemical characteristics consistent with favorable absorption profiles, with HA displaying high lipophilicity and predicted good intestinal absorption.

5.2. ADME AND TOXICOLOGICAL SAFETY PROFILE

Toxicological screening represents an essential stage in the drug development process, contributing to the selection and prioritization of molecules with the highest potential for safe and effective use in humans. This process also enhances the therapeutic properties of existing compounds and reduces the likelihood of costly late-stage failures (Tran *et al*., 2023). Prolonged exposure to chemical substances may cause various types of organ damage, including neurotoxicity, genotoxicity, immunotoxicity, carcinogenicity, and reproductive and developmental toxicity (Sabarwal *et al*., 2018).

In the present study, the web-based tools SwissADME, pkCSM, ADMETlab 3.0, and ProTox 3.0 were used to predict the drug-like properties and ADMET profiles of HA. The toxicological evaluation of HA, DZP, and FLU revealed marked differences, with HA showing the highest tolerance (LD_{50} of 900 mg/kg; Class 4), followed by FLU (LD_{50} of 1300 mg/kg; Class 4) and DZP, which exhibited the lowest tolerance (LD_{50} of 48 mg/kg; Class 2) and, consequently, the highest acute toxicity.

None of the compounds showed hepatotoxicity, carcinogenicity, immunotoxicity, or mutagenicity, suggesting overall favorable safety profiles. However, the cytotoxicity observed for DZP may compromise its safety in applications requiring high doses or prolonged use, representing a potential limitation of its therapeutic window. In contrast, HA exhibited good tolerance, with an LD_{50} of 900 mg/kg and classification in toxicity class 4, and did not show hepatotoxic, carcinogenic, immunotoxic, or mutagenic effects. Table 3 indicates that all calculated parameters for HA remained within acceptable limits.

5.3. MOLECULAR INTERACTIONS WITH GABAERGIC PATHWAYS

The GABA receptor represents the primary target for the development of anxiolytic and/or antidepressant drugs (Kalueff and Nutt, 2007). GABA is an essential inhibitory neurotransmitter involved in brain growth and function, as well as in maintaining the balance between neuronal excitation and inhibition in the CNS (Schür *et al.*, 2016). Allosteric regulation at the benzodiazepine (BDZ) binding site is one of the most widely used strategies for modulating GABAergic systems (Bappi *et al.*, 2024). However, benzodiazepines may induce tolerance and physical dependence. Moreover, conventional antidepressants exhibit limited efficacy in some patients, highlighting the urgent need for the development of new, more effective, and safer therapeutic agents (Fiedorowicz and Swartz, 2004).

Human proteins are frequently employed in molecular docking studies to support in vivo investigations using laboratory animals such as mice and zebrafish (Bhuia *et al.*, 2023; Chowdhury *et al.*, 2024; Mukty *et al.*, 2024). The primary objective of this study was to analyze the docking scores and interactions between HA, DZP, and FLU with $GABA_A$ receptor subunits (α1, α2, α3, α5, and γ2) and the human 5-HT1A receptor.

The results indicated that HA exhibited strong binding affinity (-6.0 kcal/mol) toward the α3 subunit of the $GABA_A$ receptor. In general, ligands with binding affinities greater than -6.0 kcal/mol are considered capable of forming stable interactions with their molecular targets (Hashem *et al.*, 2022; Nath *et al.*, 2021).

Furthermore, HA and DZP shared similar amino acid residues within the α3 subunit (Ala A:347, Ser A:351, Val A:344). DZP and FLU displayed higher affinities than HA, whereas HA and DZP showed comparable affinities toward the α2 subunit. Promising interactions were observed for all three compounds with the α1, α2, α3, and α5 subunits, mainly through hydrophobic interactions.

5.4. SEROTONERGIC MODULATION AND 5-HT_{1A} BINDING

Docking analyses with the 5-HT1A receptor demonstrated that fluoxetine (FLX) exhibited higher binding affinity (-7.7 kcal/mol) compared to HA (-4.7 kcal/mol), indicating stronger binding potential. This affinity is supported by diverse intermolecular interactions, including hydrogen bonds, hydrophobic interactions, π-cation interactions, and halogen bonds, which contribute to stabilization of the receptor-ligand complex. In contrast, HA showed lower affinity due to a reduced diversity of interactions.

Both compounds exhibited interactions with critical residues of the 5-HT_{1A} receptor, such as Asp A:401, Leu A:400, Phe A:320, Tyr A:107, Gly A:26, Leu A:29, Asp A:404, and Ala A:319, which are described in the literature as essential for anchoring agonists and allosteric modulators. These findings suggest that HA may mimic binding mechanisms typical of serotonergic drugs, such as fluoxetine.

The formation of stable complexes strongly depends on the presence of hydrogen bonds and hydrophobic interactions, which are fundamental for conformational stability and binding specificity. These data, together with the favorable pharmacokinetic and toxicological profile of HA, reinforce its potential as a candidate anxiolytic or antidepressant agent, justifying further in vitro and in vivo investigations to validate its functional activity.

The shared stabilizing interactions between ligands are crucial for overall receptor stability and binding affinity, highlighting the importance of hydrogen bonding in determining molecular specificity.

4. CONCLUSION

The results of this study show that hexadecanoic acid (HA), despite its high lipophilicity, has favorable pharmacokinetic characteristics, such as adequate molecular weight, good intestinal absorption, low acute toxicity and no genotoxic, carcinogenic or hepatotoxic effects. In addition, its primary metabolism by the CYP3A4 enzyme suggests a low risk of drug interactions. Although HA has limited permeability to the blood-brain barrier, molecular docking data indicate a relevant potential as a modulator of the GABAergic and serotoninergic pathways, with significant affinity for $GABA_A$ and $5\text{-}HT_{1A}$ receptors. The compound formed stable interactions at the binding sites, mediated mainly by hydrophobic and van der Waals forces. The stability of the HA-receptor complexes, together with the interaction with key residues of the active site, suggests that HA can modulate the activity of these receptors, with possible therapeutic applications in the treatment of anxiety and depression-related disorders. This evidence positions HA as a promising candidate for future in vivo investigations aimed at validating its anxiolytic potential and exploring its viability as a therapeutic agent. Further studies are needed to evaluate its efficacy in animal and clinical models, as well as to develop strategies that overcome its limited penetration into the central nervous system, such as the use of controlled release systems or nanotechnology.

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FINANCIAL INTERESTS

The authors declare that they have no conflict of interest.

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